```
  1  TTTCCTCACTGACTATAAAAGAATAGAGAAGGAAGGGCTTCAGTGACCGGCTGCCTGGCTGACTTACAGCAGTCAGACTCTGACAGGATC

  1  ATGGCTATGATGGAGGTCCAGGGGGGACCCAGCCTGGGACAGACCTGCGTGCTGATCGTGATCTTCACAGTGCTCCTGCAGTCTCTCTGT
  1  MetAlaMetMetGluValGlnGlyGlyProSerLeuGlyGlnThrCysValLeuIleValIlePheThrValLeuLeuGlnSerLeuCys

181  GTGGCTGTAACTTACGTGTACTTTACCAACGAGCTGAAGCAGATGCAGGACAAGTACTCCAAAAGTGGCATTGCTTGTTTCTTAAAAGAA
 31  ValAlaValThrTyrValTyrPheThrAsnGluLeuLysGlnMetGlnAspLysTyrSerLysSerGlyIleAlaCysPyeLeuL6sGlu

271  GATGACAGTTATTGGGACCCCAATGACGAAGAGAGTATGAACAGCCCCTGCTGGCAAGTCAAGTGGCAACTCCGTCAGCTCGTTAGAAAG
 61  AspAspSerTyrTrpAspProAsnAspGluGluSerMetAsnSerProCysTrpGlnValLysTrpGlnLeuArgGlnLeuValArgLys

361  ATGATTTTGAGAACCTCTGAGGAAACCATTTCTACAGTTCAAGAAAAGCAACAAAATATTTCTCCCCTAGTGAGAGAAAGAGGTCCNCAG
 91  MetIleLeuArgThrSerGluGluThrIleSerThrValGlnGluLysGlnGlnAsnIleSerProLeuValArgGluArgGlyProGln
                                                                *
451  AGAGTAGCAGCTCACATAACTGGGACCAGAGGAAGAAGCAACACATTGTCTTCTCCAAACTCCAAGAATGAAAAGGCTCTGGGCCGCAAA
121  ArgValAlaAlaHisIlethrGlyThrArgGlyArgSerAsnThrLeuSerSerProAsnSerLysAsnGluLysAlaLeuGlyArgLys

541  ATAAACTCCTGGGAATCATCAAGGAGTGGGCATTCATTCCTGAGCAACTTGCACTTGAGGAATGGTGAACTGGTCATCCATGAAAAAGGG
151  IleAsnSerTrpGluSerSerArgSerGlyHisSerPheLeuSerAsnLeuHisLeuArgAsnGlyGluLeuValIleHisGluLysGly

631  TTTTACTACATCTATTCCCAAACATACTTTCGATTTCAGGAGGAAATAAAAGAAAACACAAAGAACGACAAACAAATGGTCCAATATATT
181  PheTyrTyrIleTyrSerGlnthrTyrPheArgPheGlnGluGluIleLysGluAsnThrLysAsnAspLysGlnMetValGlnTyrIle

721  TACAAATACACAAGTTATCCTGACCCTATATTGTTGATGAAAAGTGCTAGAAATAGTTGTTGGTCTAAAGATGCAGAATATGGACTCTAT
211  TyrLysTyrThrSerTyrProAspProIleLeuLeuMetLysSerAlaArgAsnSerCysTrpSerLysAspAlaGluTyrGlyLeuTyr

811  TCCATCTATCAAGGGGGAATATTTGAGCTTAAGGAAAATGACAGAATTTTTGTTTCTGTAACAAATGAGCACTTGATAGACATGGACCAT
241  SerIleTyrGlnGlyGlyIlePheGluLeuLysGluAsnAspArgIlePheValSerValThrAsnGluHisLeuIleAspMetAspHis

901  GAAGCCAGTTTTTTCGGGGCCTTTTTAGTTGGCTAACTGACCTGGAAAGAAAAAGCAATAACCTCAAAGTGACTATTCAGTTTTCAGGAT
271  GluAlaSerPhePheGlyAlaPheLeuValGlyStp

991  GATACACTATGAAGATGTTTCAAAAAATCTGACCAAAACAAACAAACAGAAA
```

FIG._1

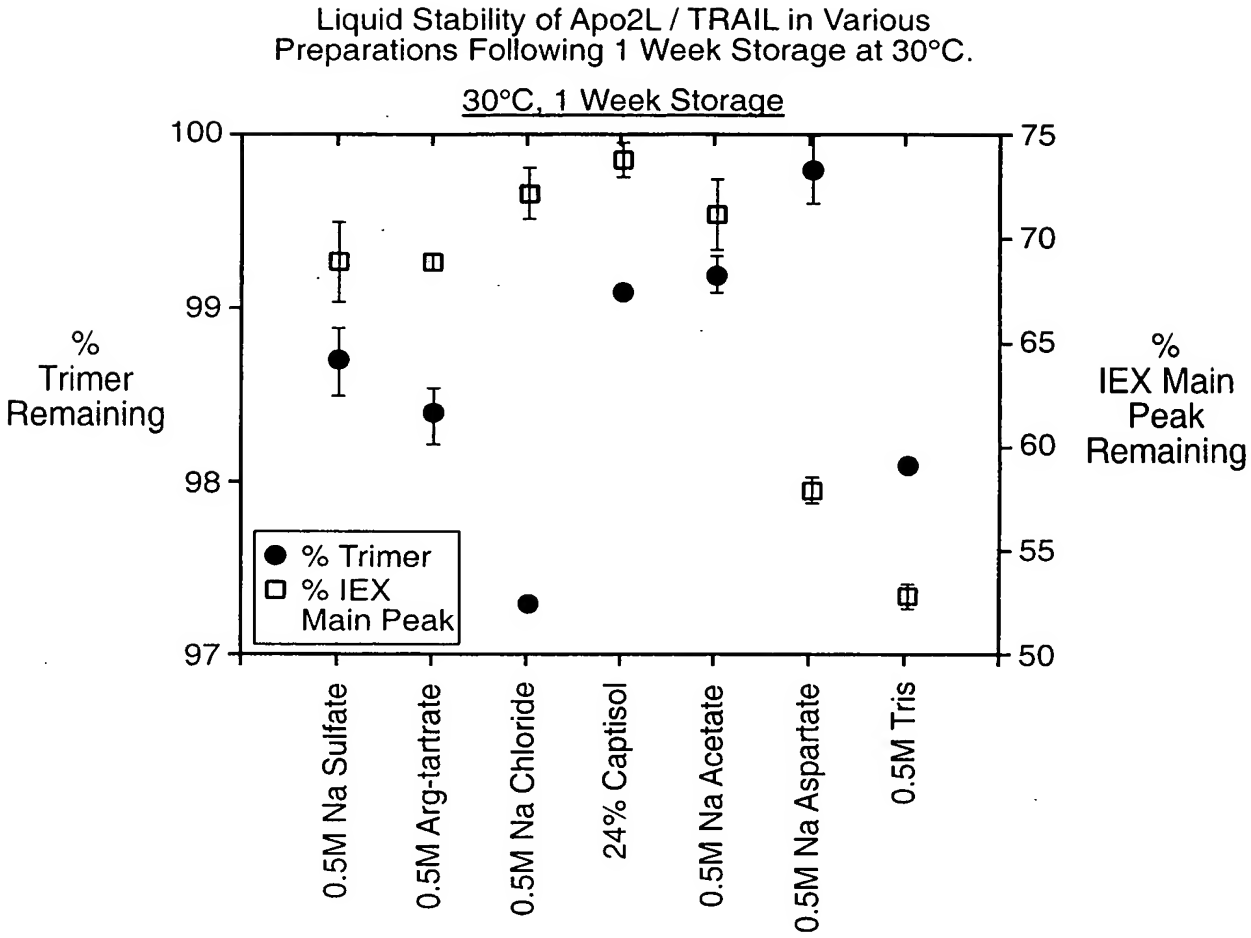

Liquid Stability of Apo2L / TRAIL in Various Preparations Following 1 Week Storage at 30°C.
30°C, 1 Week Storage
% Trimer Remaining
% IEX Main Peak Remaining
100
99
98
97
75
70
65
60
55
50
% Trimer
% IEX Main Peak
0.5M Na Sulfate
0.5M Arg-tartrate
0.5M Na Chloride
24% Captisol
0.5M Na Acetate
0.5M Na Aspartate
0.5M Tris

FIG._2

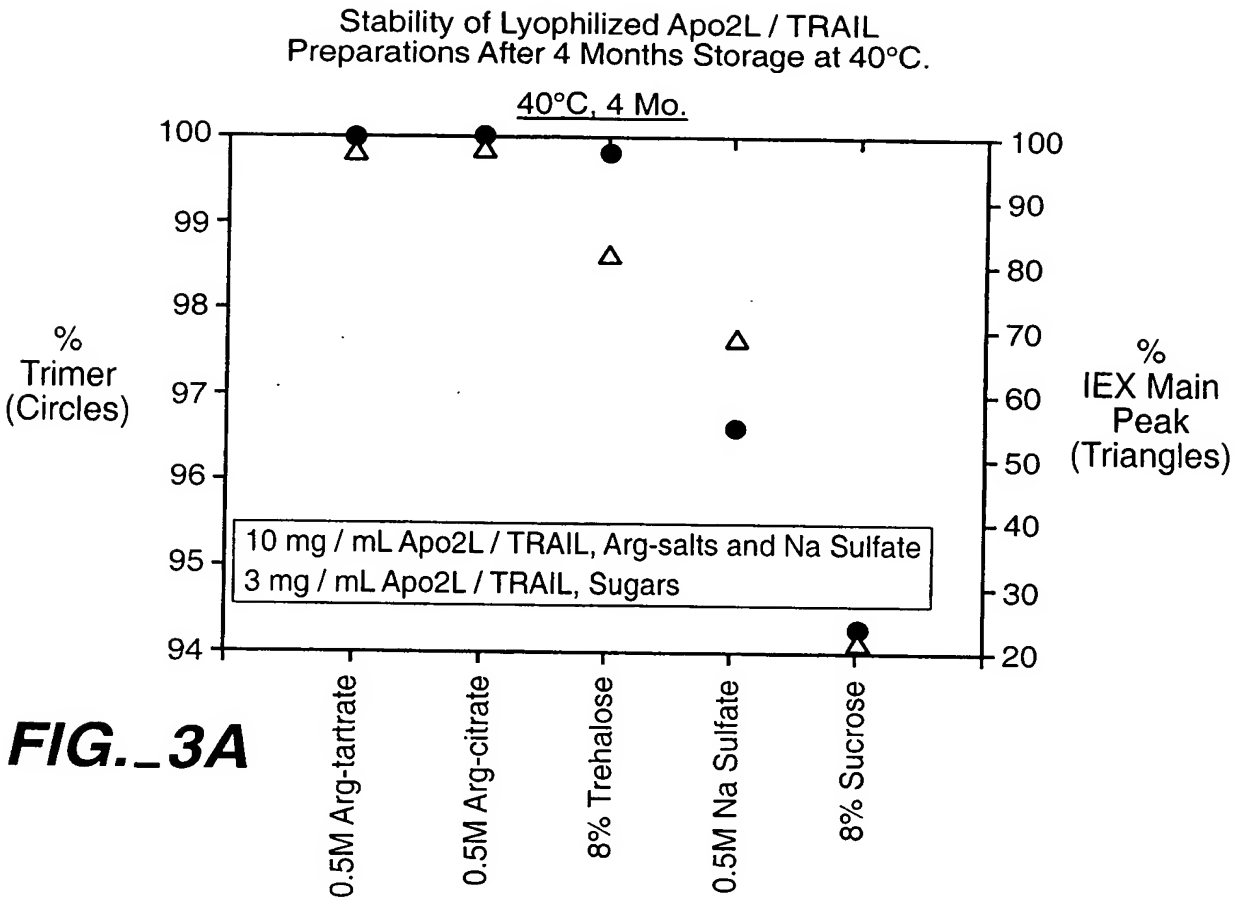

Stability of Lyophilized Apo2L / TRAIL
Preparations After 4 Months Storage at 40°C.
40°C, 4 Mo.
100
99
98
97
96
95
94
% Trimer (Circles)
100
90
80
70
60
50
40
30
20
% IEX Main Peak (Triangles)
10 mg / mL Apo2L / TRAIL, Arg-salts and Na Sulfate
3 mg / mL Apo2L / TRAIL, Sugars
0.5M Arg-tartrate
0.5M Arg-citrate
8% Trehalose
0.5M Na Sulfate
8% Sucrose

FIG._3A

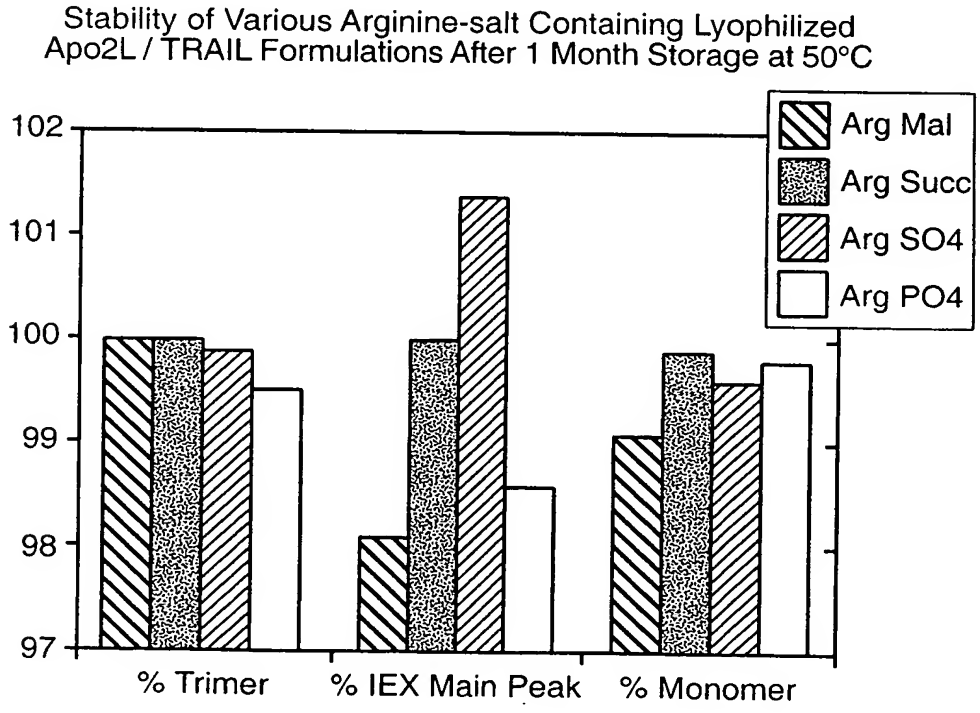

Stability of Various Arginine-salt Containing Lyophilized
Apo2L / TRAIL Formulations After 1 Month Storage at 50°C
102
101
100
99
98
97
Arg Mal
Arg Succ
Arg SO4
Arg PO4
% Trimer
% IEX Main Peak
% Monomer

FIG._3B

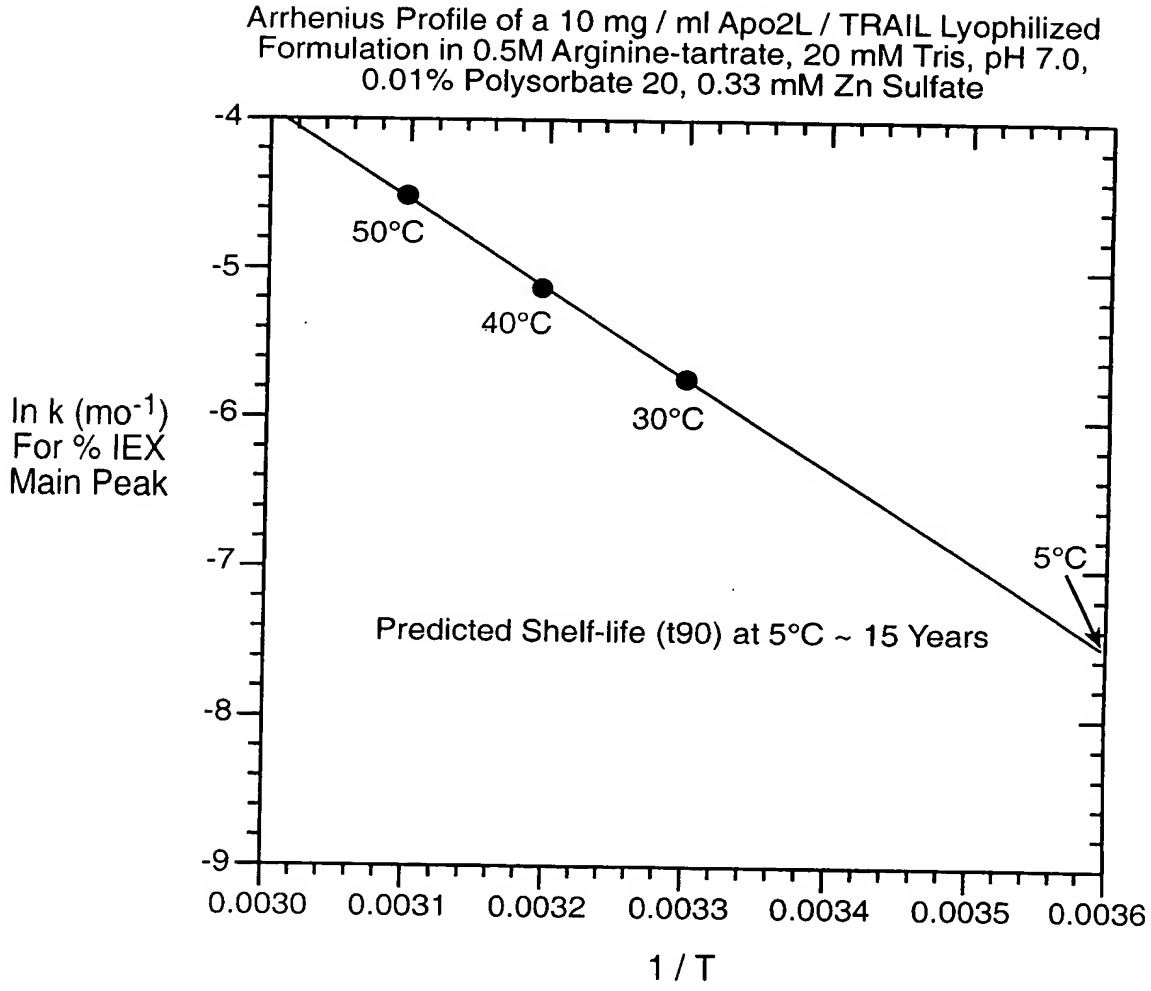

Arrhenius Profile of a 10 mg / ml Apo2L / TRAIL Lyophilized Formulation in 0.5M Arginine-tartrate, 20 mM Tris, pH 7.0, 0.01% Polysorbate 20, 0.33 mM Zn Sulfate
ln k (mo-1) For % IEX Main Peak
-4
-5
-6
-7
-8
-9
50°C
40°C
30°C
5°C
Predicted Shelf-life (t90) at 5°C ~ 15 Years
0.0030
0.0031
0.0032
0.0033
0.0034
0.0035
0.0036
1 / T

FIG._3C

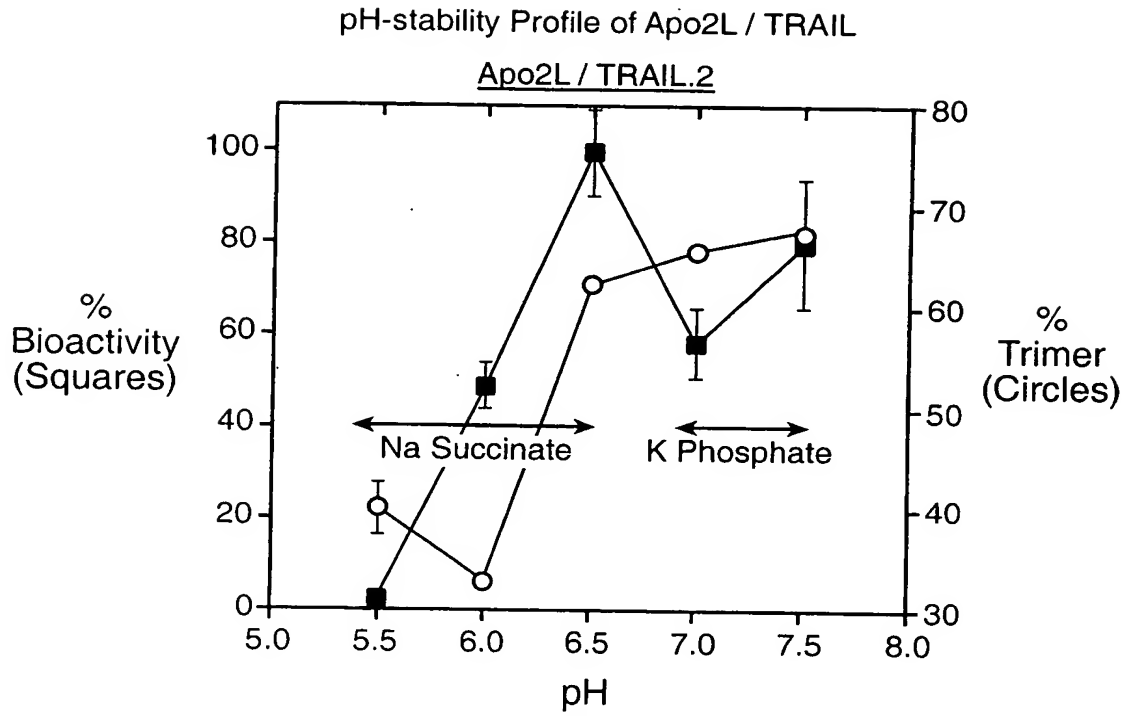

pH-stability Profile of Apo2L / TRAIL
Apo2L / TRAIL.2
% Bioactivity (Squares)
% Trimer (Circles)
Na Succinate
K Phosphate
pH
100
80
60
40
20
0
80
70
60
50
40
30
5.0
5.5
6.0
6.5
7.0
7.5
8.0

FIG._4A

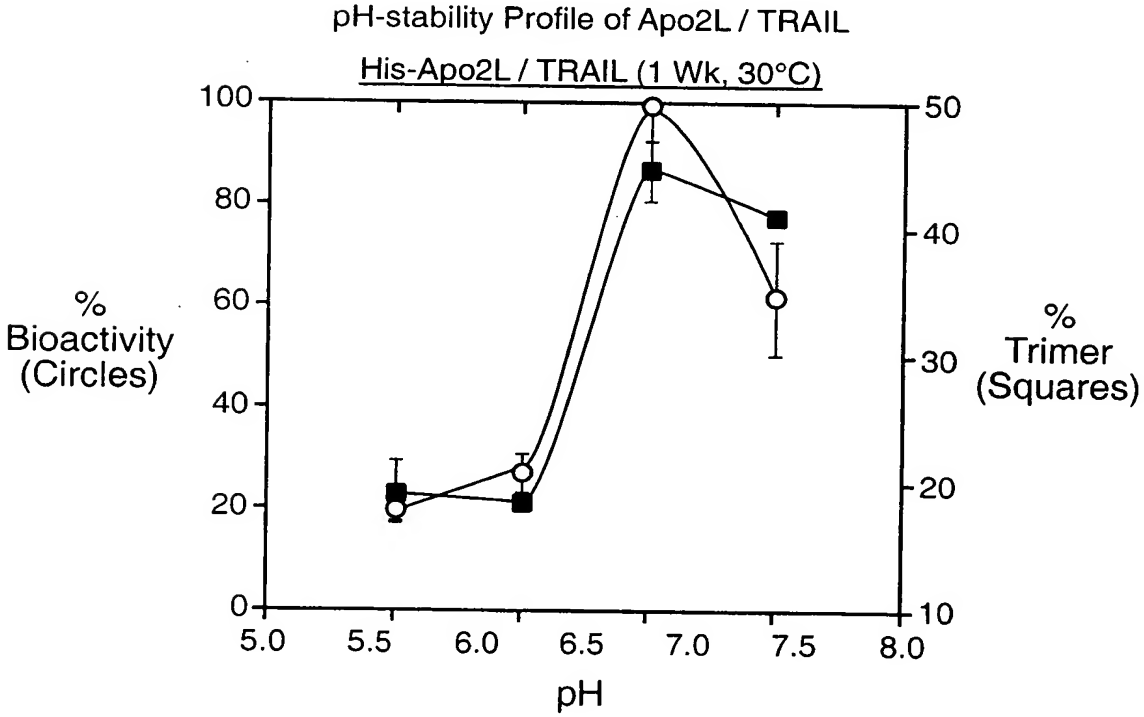

pH-stability Profile of Apo2L / TRAIL
His-Apo2L / TRAIL (1 Wk, 30°C)
% Bioactivity (Circles)
% Trimer (Squares)
pH
100
80
60
40
20
0
50
40
30
20
10
5.0
5.5
6.0
6.5
7.0
7.5
8.0

FIG._4B

Zn Coordination to Apo2L / Trail and Effect of pH

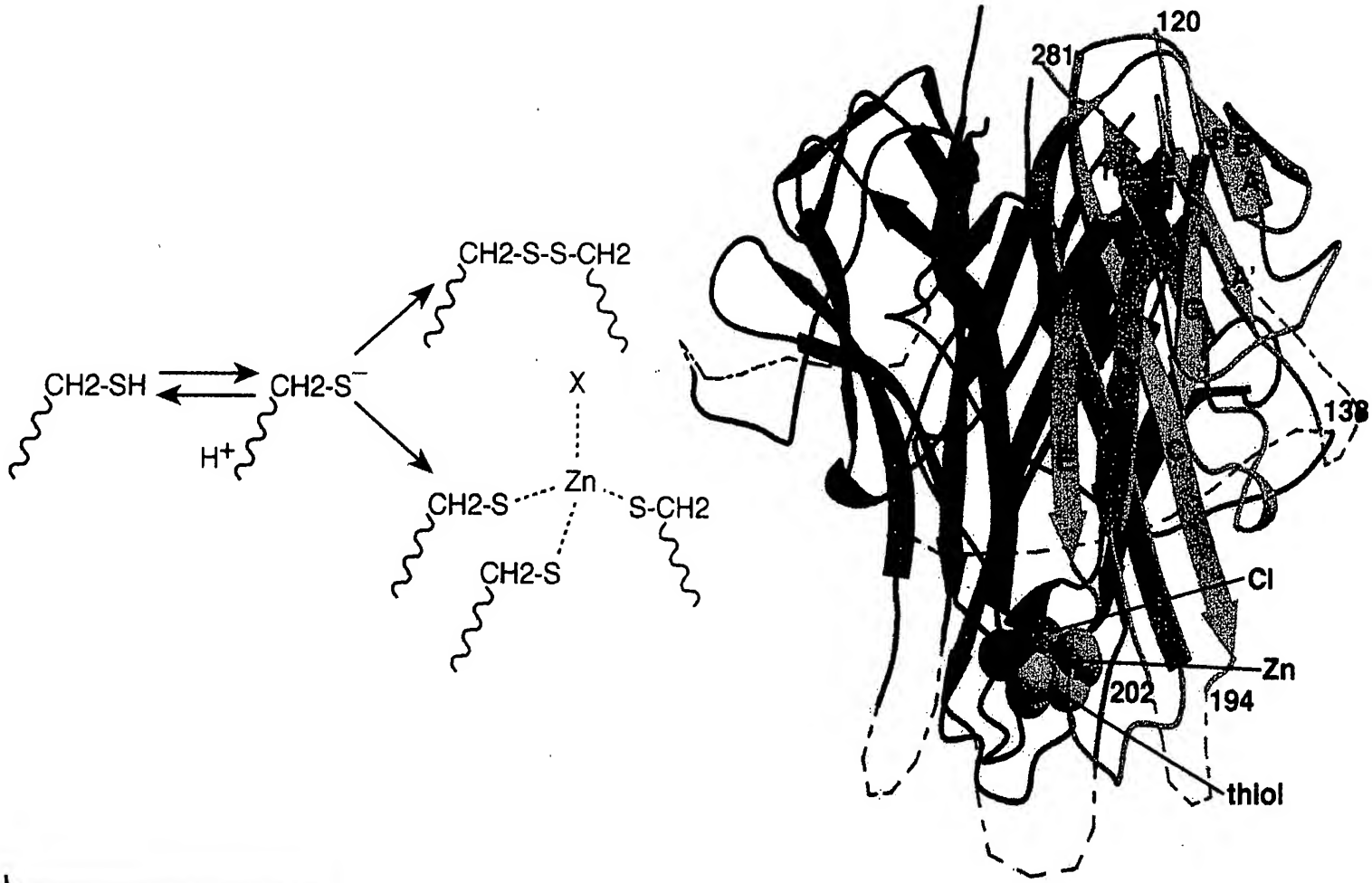


FIG._5

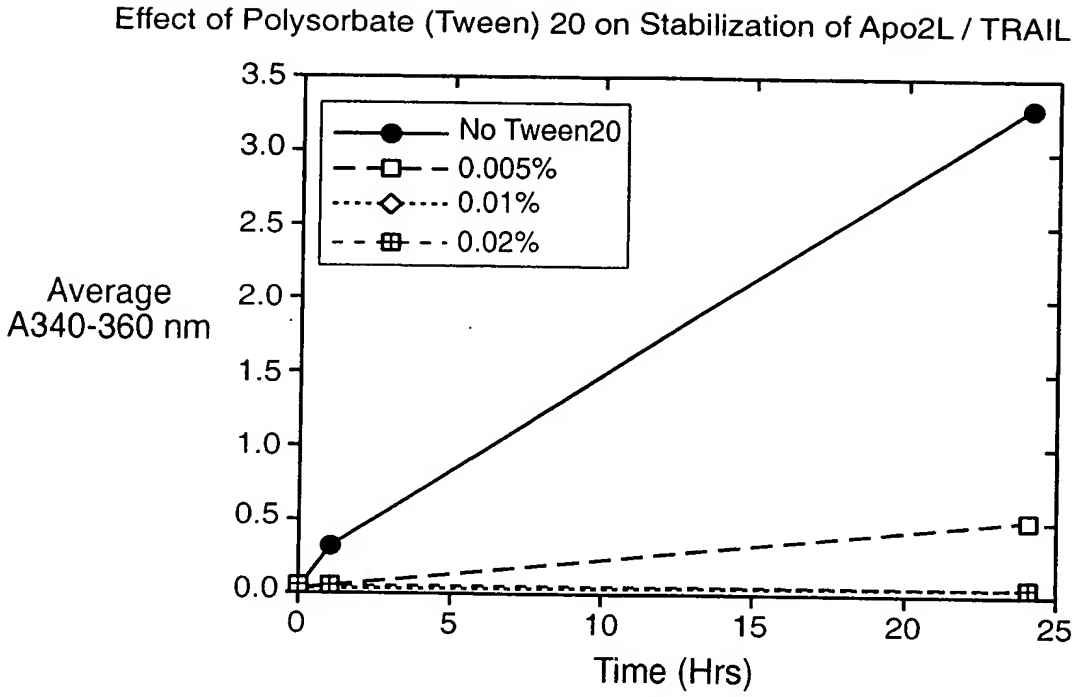

Effect of Polysorbate (Tween) 20 on Stabilization of Apo2L / TRAIL
Average A340-360 nm
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
No Tween20
0.005%
0.01%
0.02%
0
5
10
15
20
25
Time (Hrs)

FIG._6

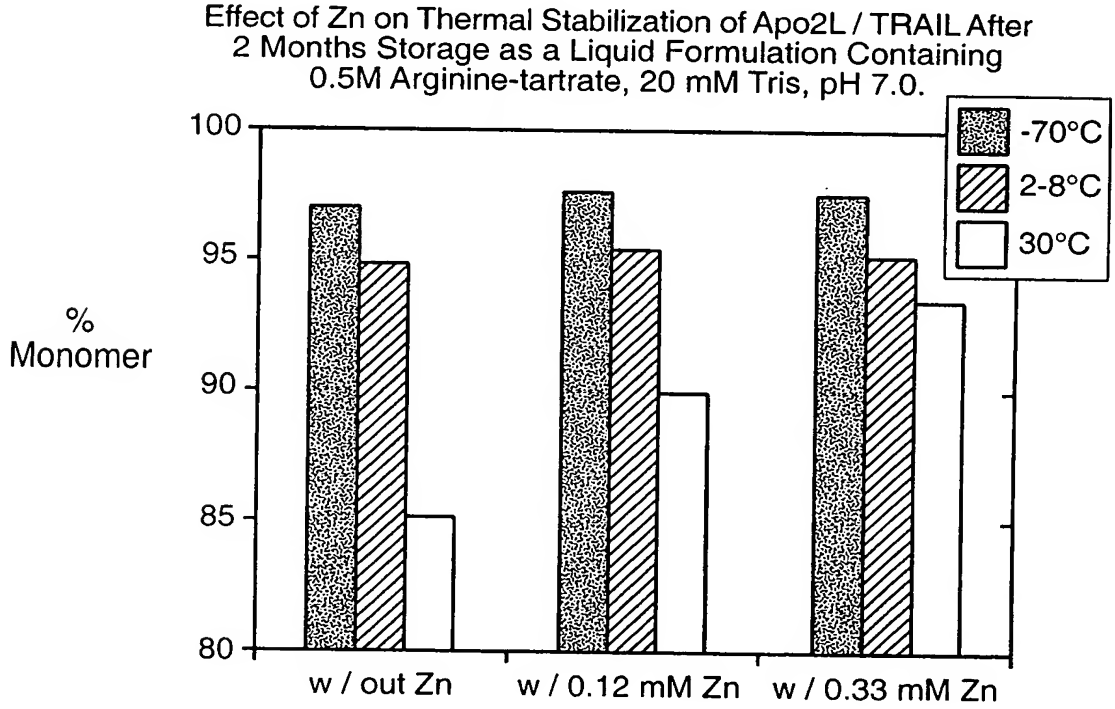
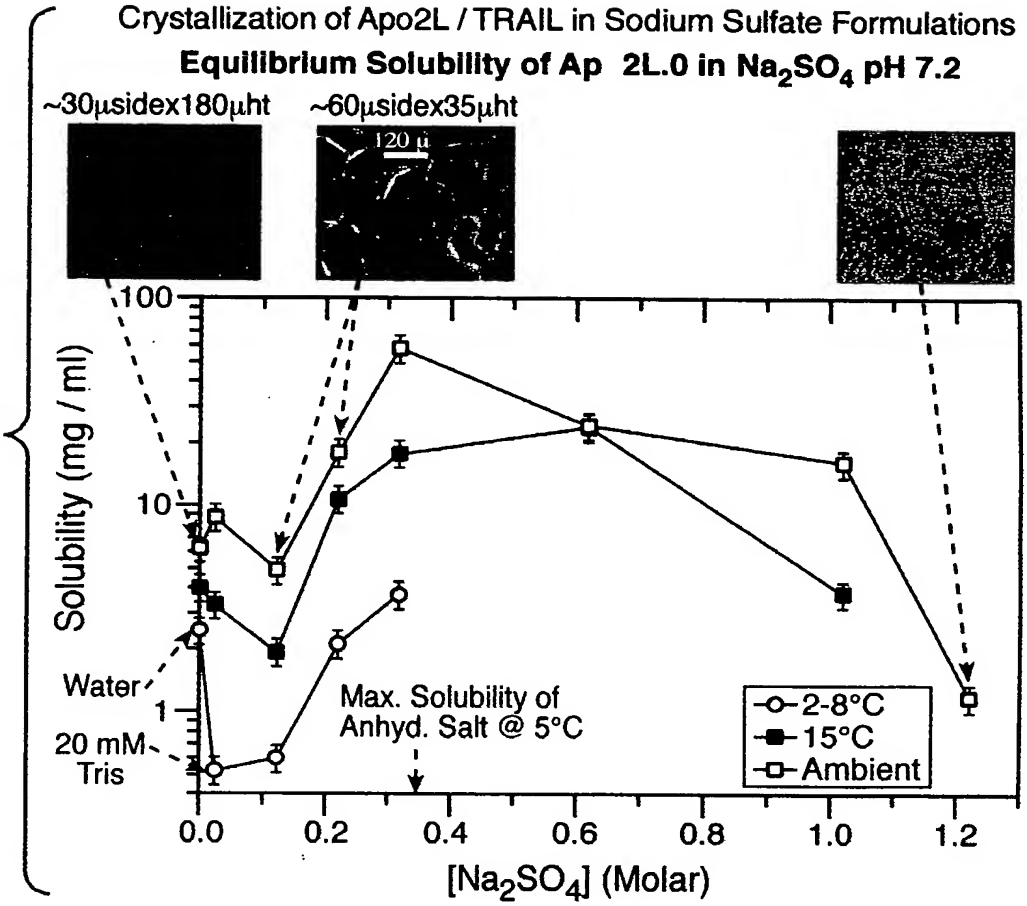

Effect of Zn on Thermal Stabilization of Apo2L / TRAIL After 2 Months Storage as a Liquid Formulation Containing 0.5M Arginine-tartrate, 20 mM Tris, pH 7.0.
% Monomer
100
95
90
85
80
-70°C
2-8°C
30°C
w / out Zn
w / 0.12 mM Zn
w / 0.33 mM Zn

FIG._7

FIG._8



Ion Dependence Crystallization of Apo2L / TRAIL. Crystallization Was Observed in All Salts, but Arginine and Mg Salts Maximized the Protein Solubility in the Narrow Range of 10-11.5 mS / cm Conductivity.

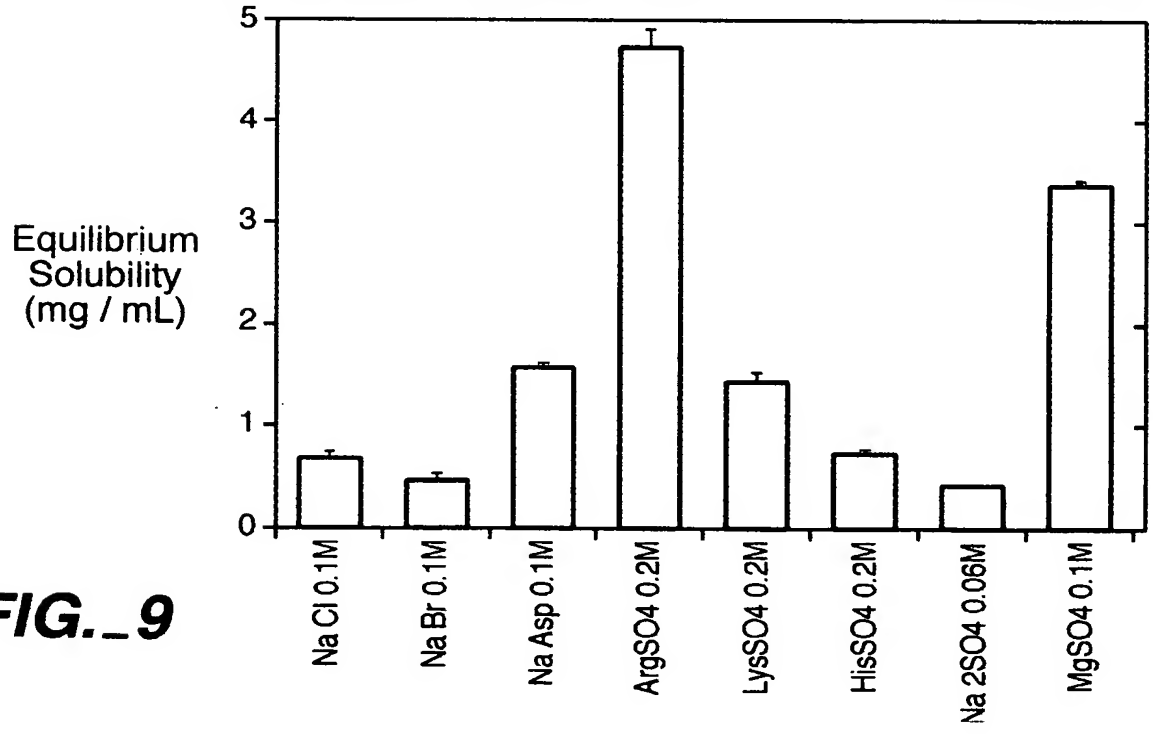


FIG._9

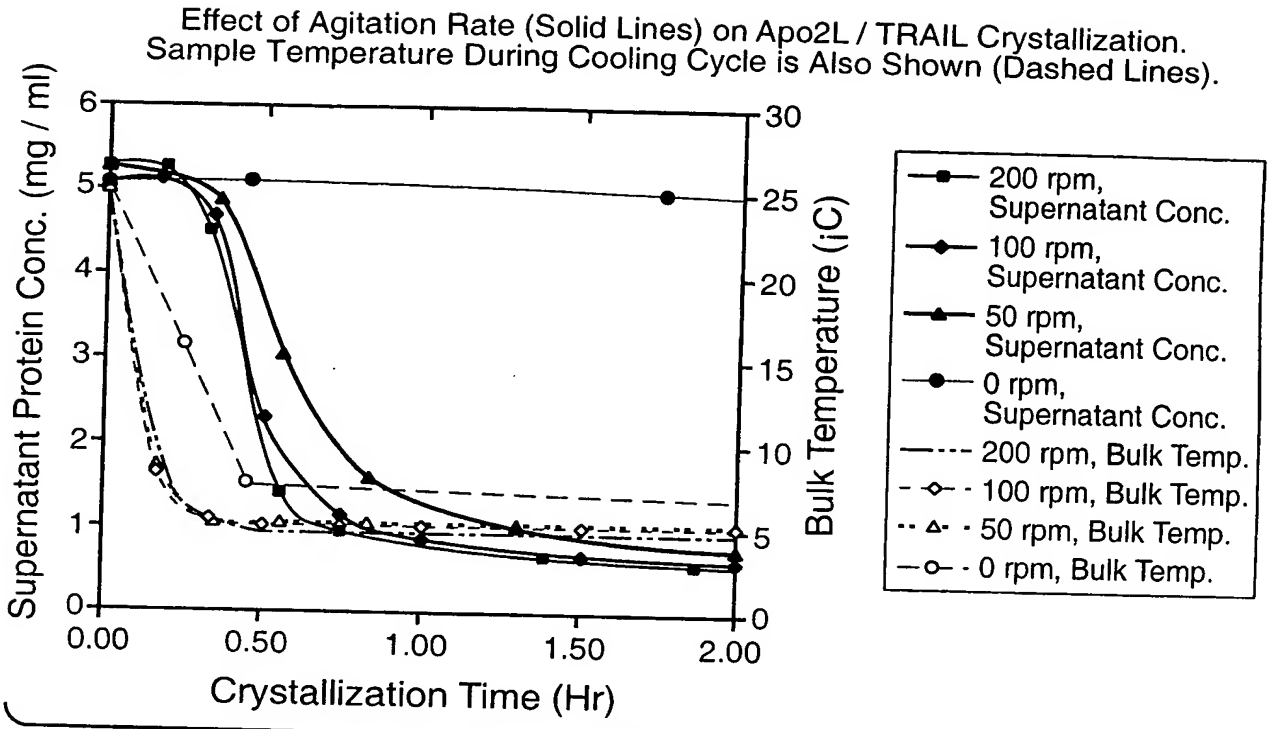


FIG._10A

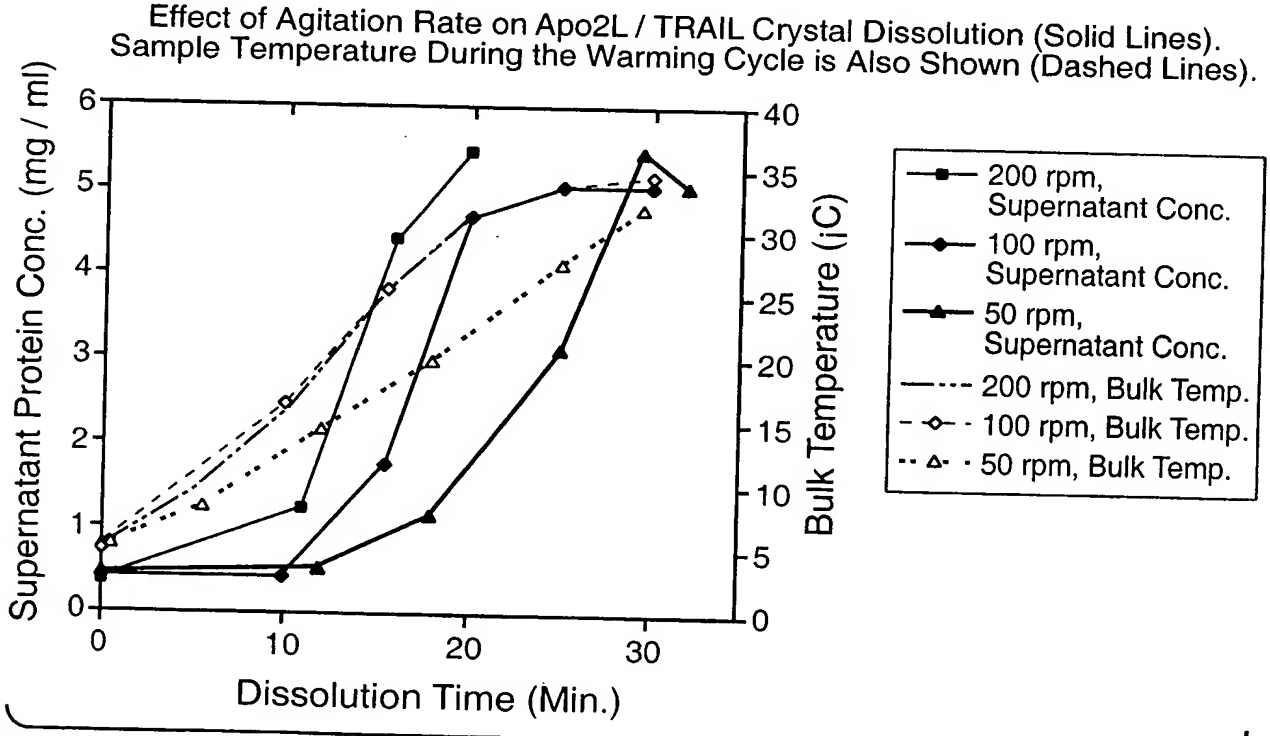


FIG._10B

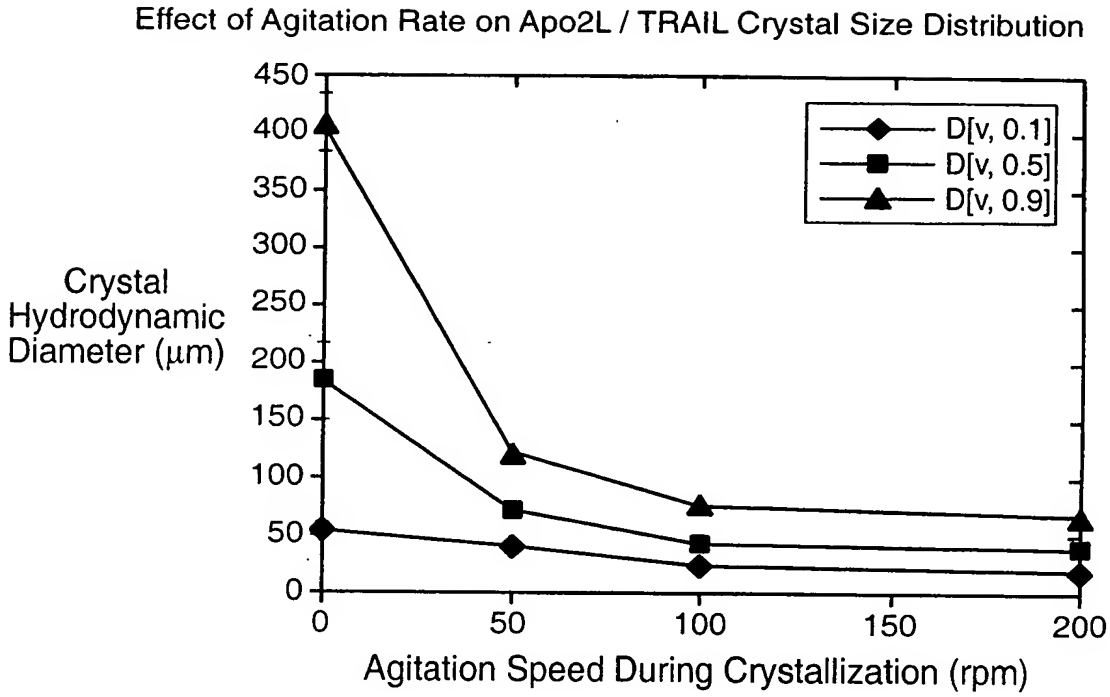


FIG._10C

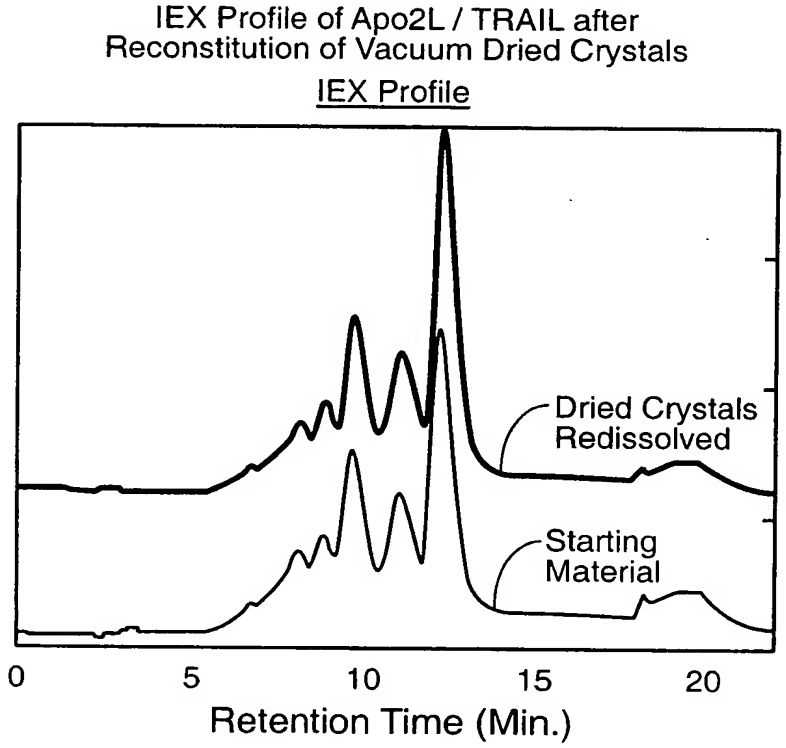


FIG._11A

Bioactivity of Apo2L / TRAIL after Reconstitution of Vacuum Dried Crystals.

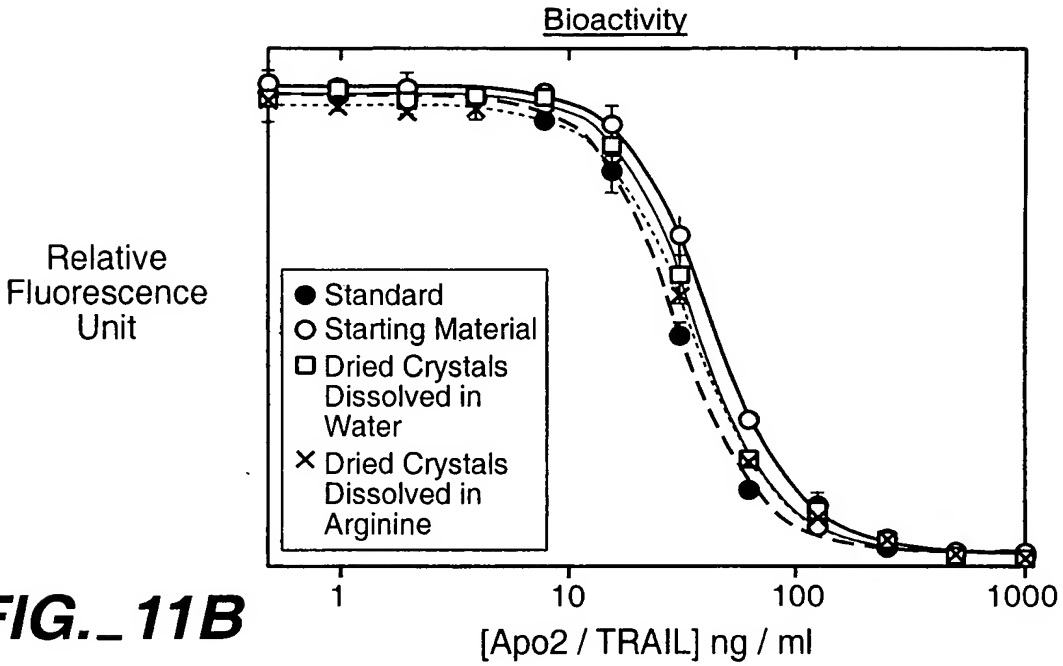


FIG._11B

Arrhenius Profile of a 20 mg / ml Apo2L / TRAIL Lyophilized Formulation in 0.2M Na Sulfate, 20 mM Tris, pH 7.2, 0.01 % tween 20.

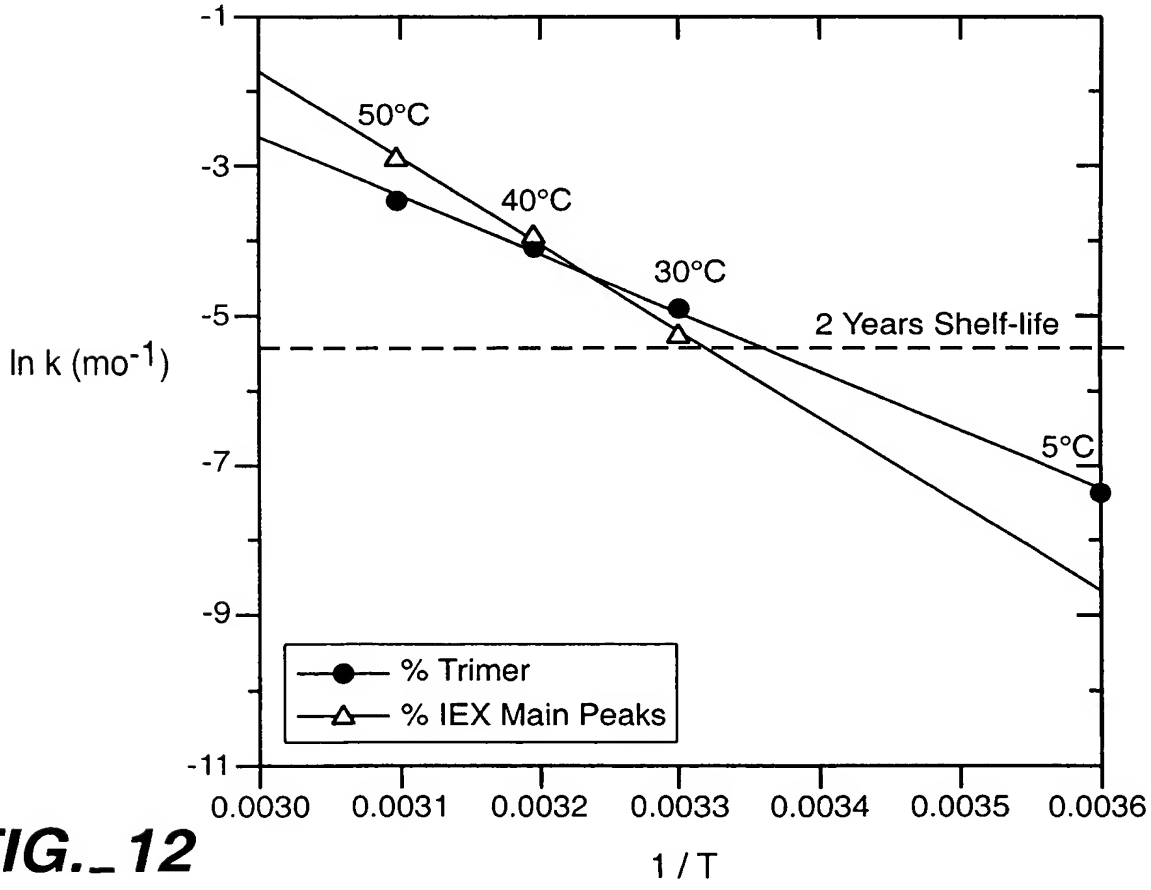


FIG._12

SDS-PAGE Silver Stain Purity of Apo2L / TRAIL Purified by Crystallization Compared to a Three-column Step Purified Reference Material.

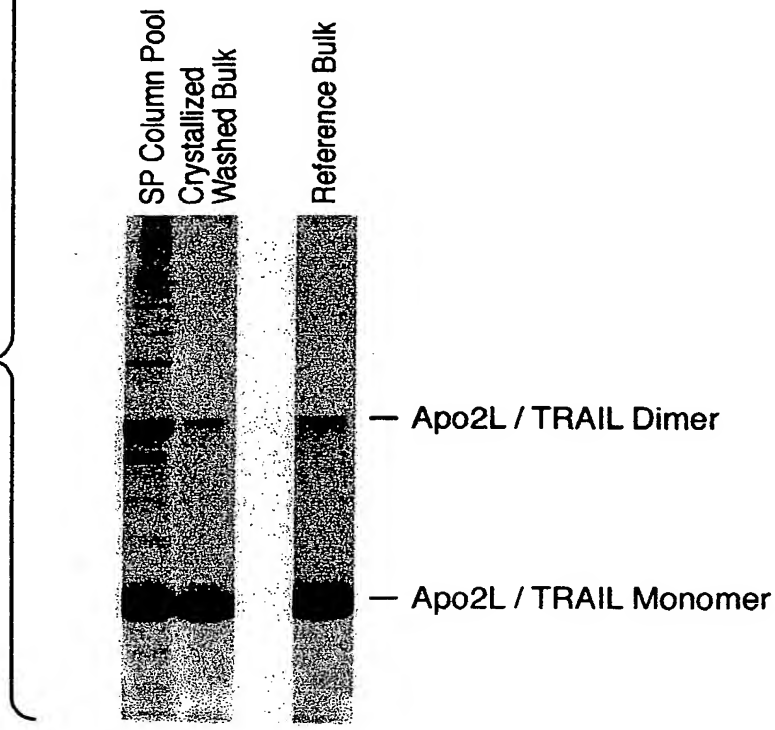


FIG._13

Effect of Salt Type on Crystallization of Partially Purified Apo2L / TRAIL. After Partial Purification of E. Coli Clarified Lysates on Sp-sepharose Cation Exchange Column, the Protein Was Eluted At 5-10 mg / ml in 20 mM Tris, pH 8 and 0.2M of One of the Salts Shown. The Samples Were Stored At 2-8°C For 3-7 Days. An Aliquot was Then Filtered and the Soluble Protein Concentration was Measured by UV Spec Scan.

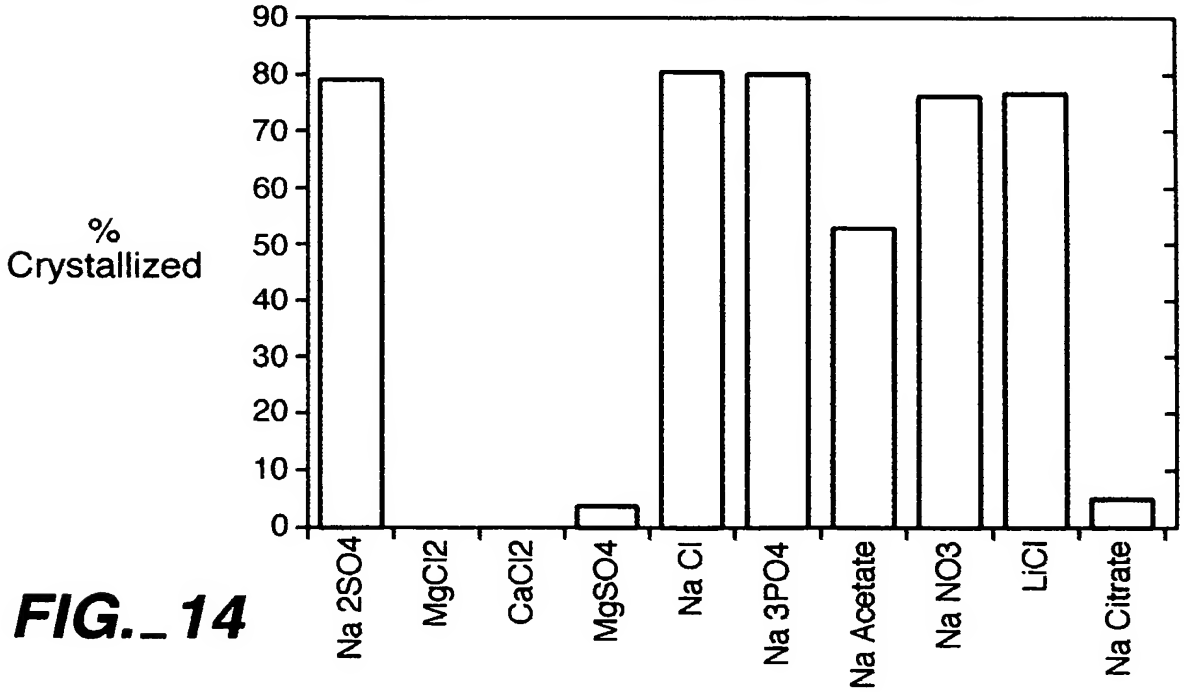


FIG._14

Figure 15A

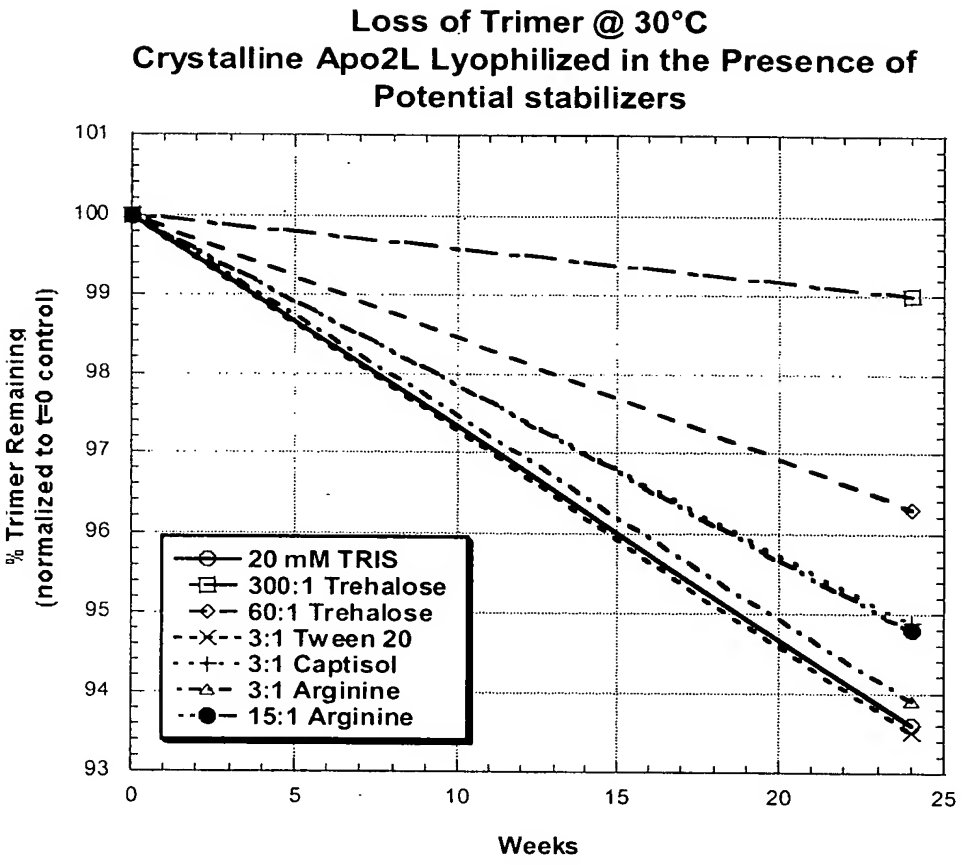

Loss of Trimer @ 30°C
Crystalline Apo2L Lyophilized in the Presence of
Potential stabilizers
% Trimer Remaining
(normalized to t=0 control)
101
100
99
98
97
96
95
94
93
0
5
10
15
20
25
Weeks
20 mM TRIS
300:1 Trehalose
60:1 Trehalose
3:1 Tween 20
3:1 Captisol
3:1 Arginine
15:1 Arginine

Figure 15B

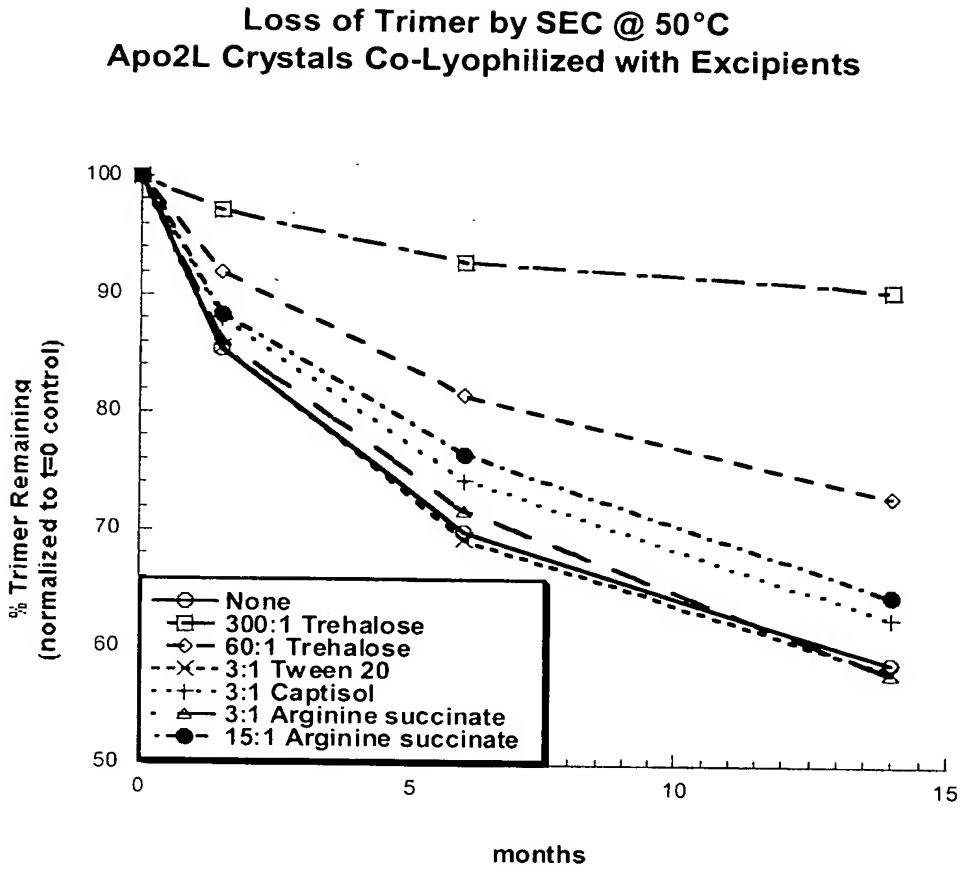

Loss of Trimer by SEC @ 50°C
Apo2L Crystals Co-Lyophilized with Excipients
% Trimer Remaining
(normalized to t=0 control)
100
90
80
70
60
50
0
5
10
15
months
None
300:1 Trehalose
60:1 Trehalose
3:1 Tween 20
3:1 Captisol
3:1 Arginine succinate
15:1 Arginine succinate

Figure 15C

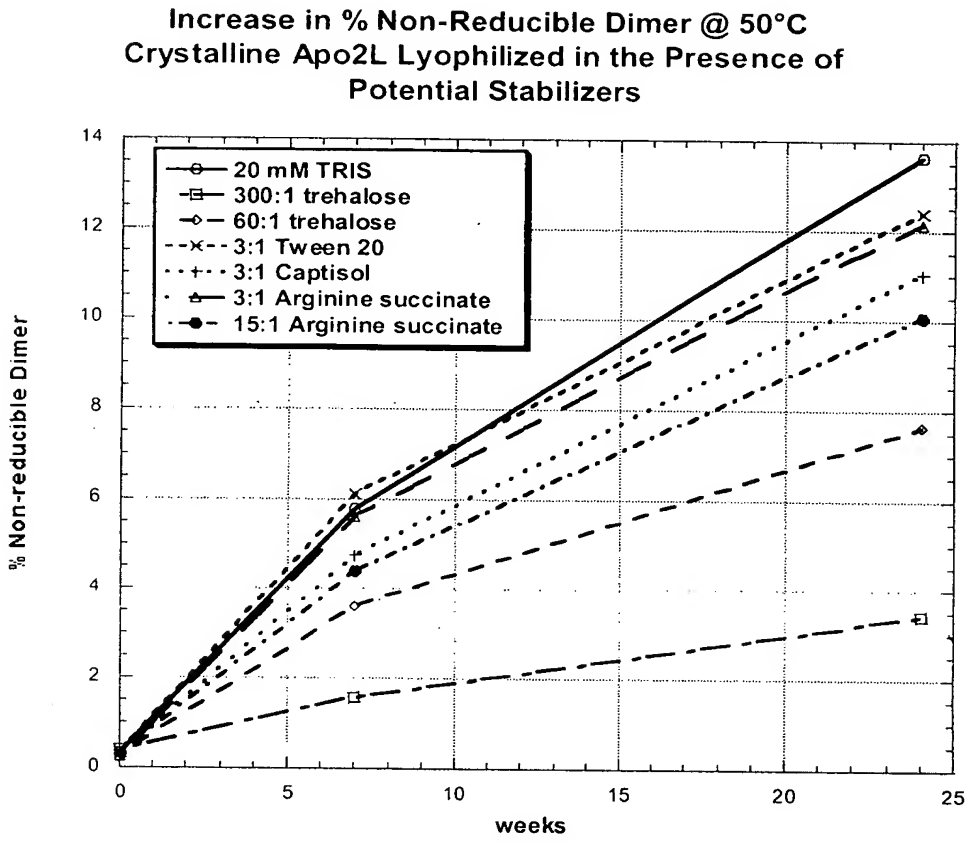

Increase in % Non-Reducible Dimer @ 50°C
Crystalline Apo2L Lyophilized in the Presence of
Potential Stabilizers
% Non-reducible Dimer
weeks
20 mM TRIS
300:1 trehalose
60:1 trehalose
3:1 Tween 20
3:1 Captisol
3:1 Arginine succinate
15:1 Arginine succinate
0
2
4
6
8
10
12
14
0
5
10
15
20
25

Figure 16A

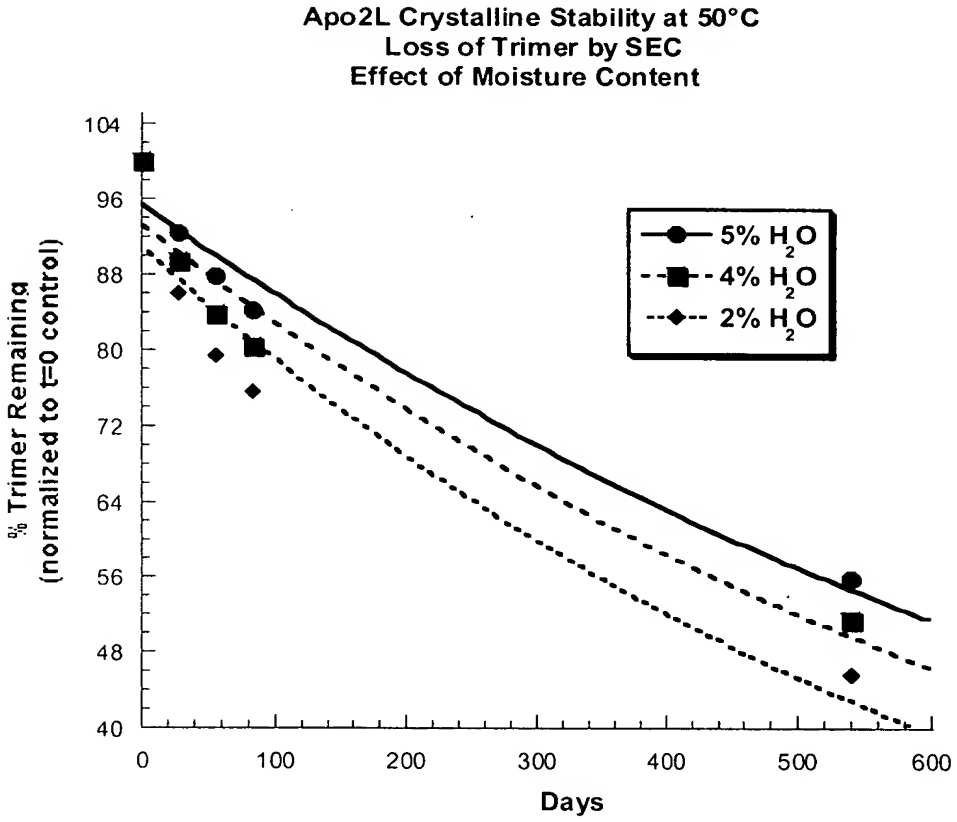

Apo2L Crystalline Stability at 50°C
Loss of Trimer by SEC
Effect of Moisture Content
% Trimer Remaining
(normalized to t=0 control)
104
96
88
80
72
64
56
48
40
0
100
200
300
400
500
600
Days
5% H2O
4% H2O
2% H2O

Figure 16B

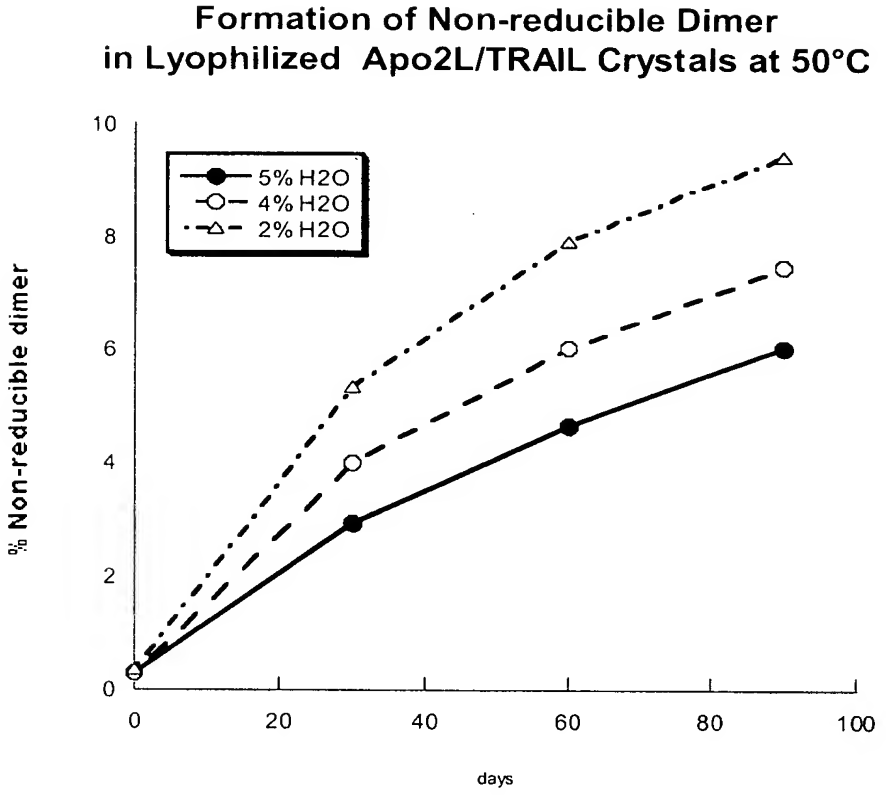

Formation of Non-reducible Dimer
in Lyophilized Apo2L/TRAIL Crystals at 50°C
% Non-reducible dimer
10
8
6
4
2
0
5% H2O
4% H2O
2% H2O
0
20
40
60
80
100
days

Figure 17A

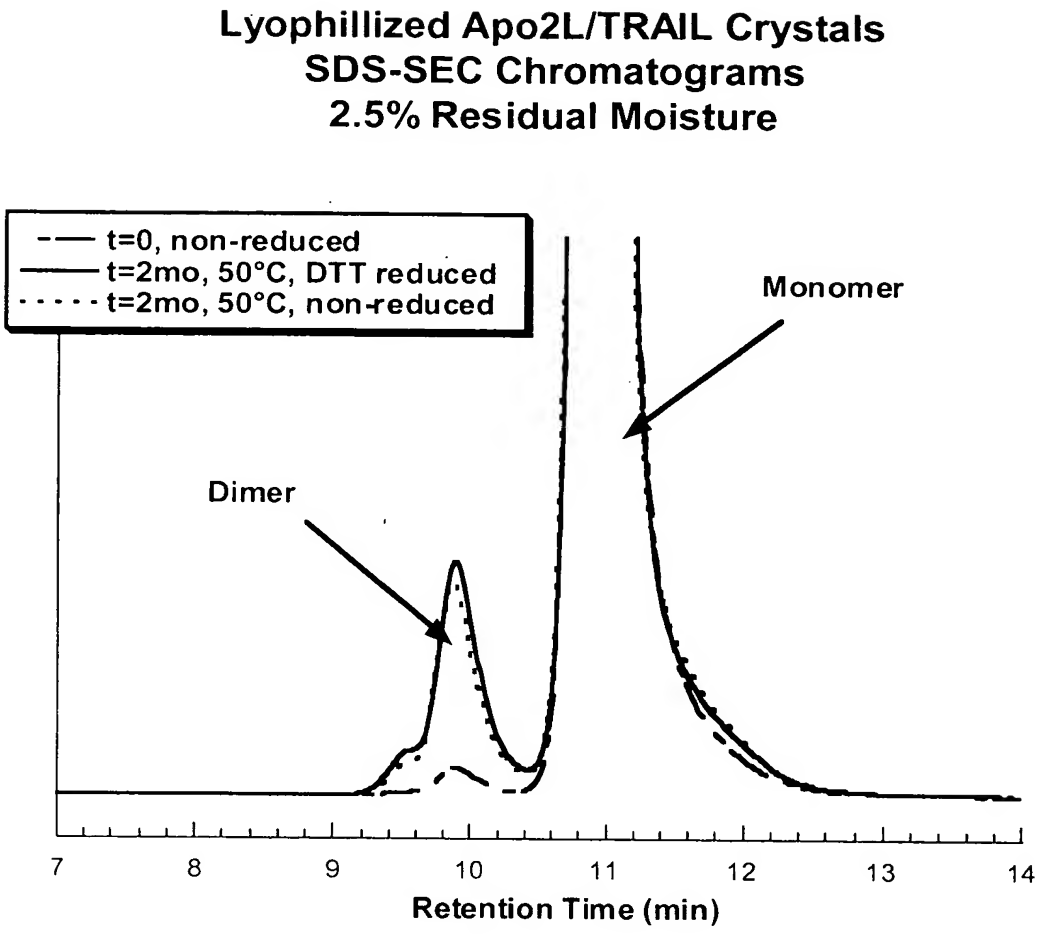

Lyophillized Apo2L/TRAIL Crystals
SDS-SEC Chromatograms
2.5% Residual Moisture
t=0, non-reduced
t=2mo, 50°C, DTT reduced
t=2mo, 50°C, non-reduced
Monomer
Dimer
7
8
9
10
11
12
13
14
Retention Time (min)

Figure 17B

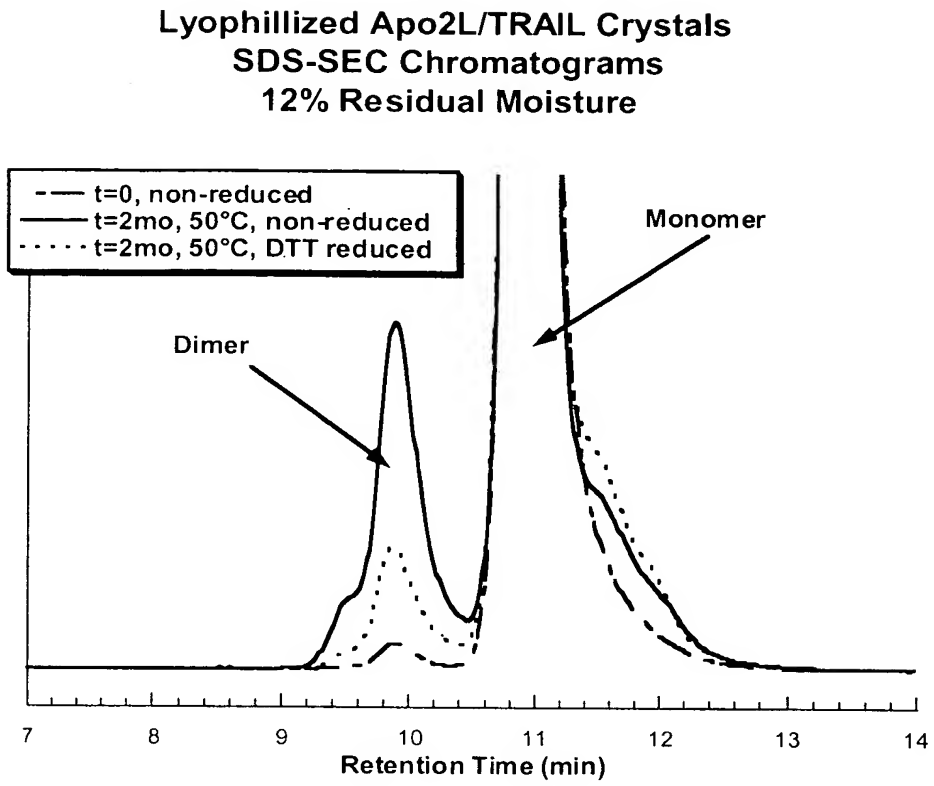

Lyophillized Apo2L/TRAIL Crystals
SDS-SEC Chromatograms
12% Residual Moisture
t=0, non-reduced
t=2mo, 50°C, non-reduced
t=2mo, 50°C, DTT reduced
Dimer
Monomer
7
8
9
10
11
12
13
14
Retention Time (min)

Figure 18A

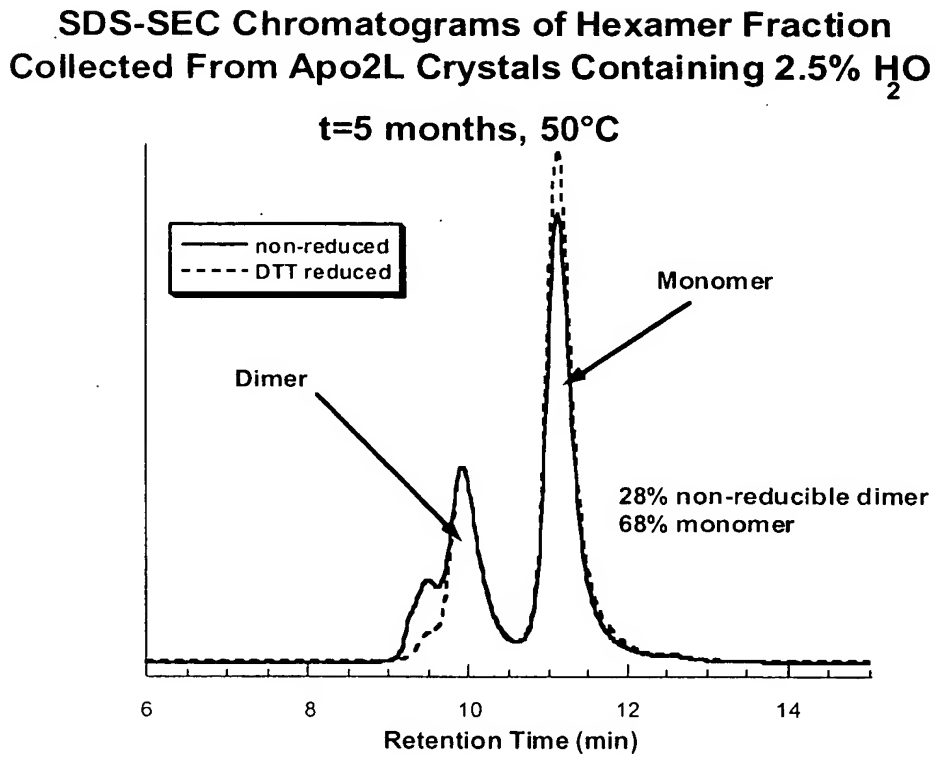

SDS-SEC Chromatograms of Hexamer Fraction
Collected From Apo2L Crystals Containing 2.5% H_2O
t=5 months, 50°C
non-reduced
DTT reduced
Monomer
Dimer
28% non-reducible dimer
68% monomer
6
8
10
12
14
Retention Time (min)

Figure 18B

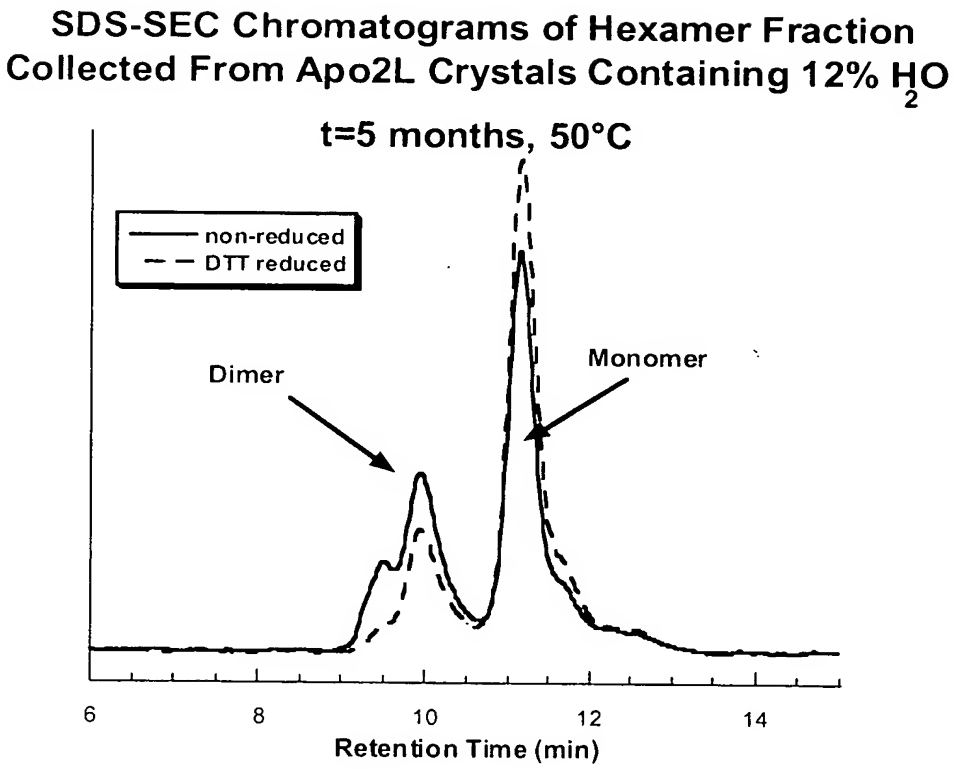

SDS-SEC Chromatograms of Hexamer Fraction Collected From Apo2L Crystals Containing 12% H_2O
t=5 months, 50°C
non-reduced
DTT reduced
Dimer
Monomer
6
8
10
12
14
Retention Time (min)

Figure 19A

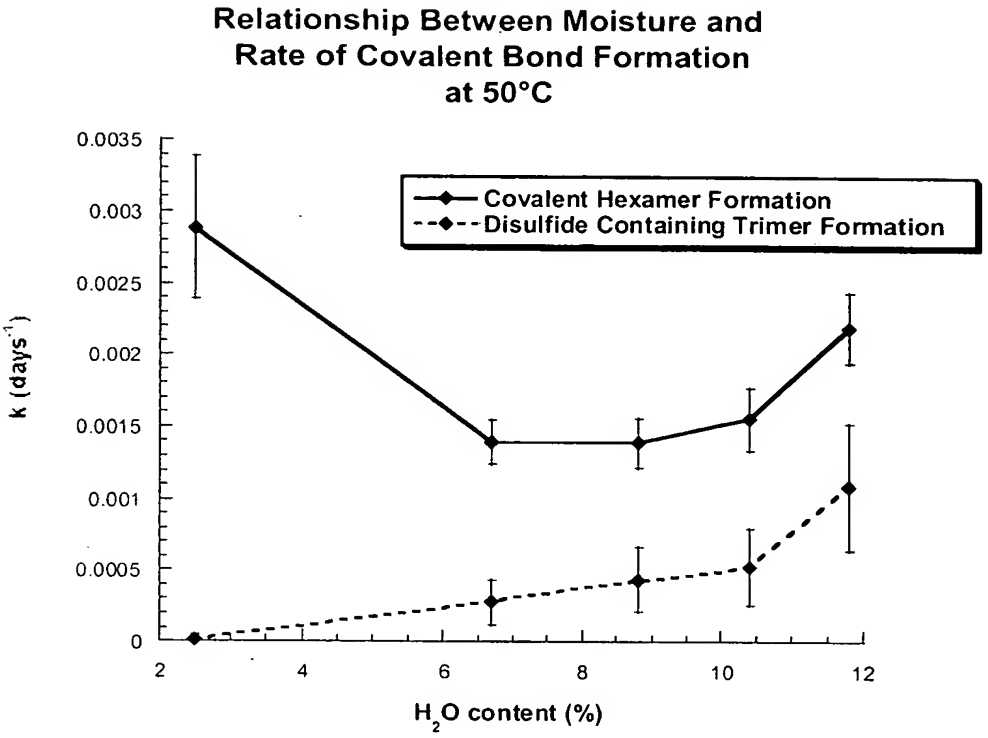
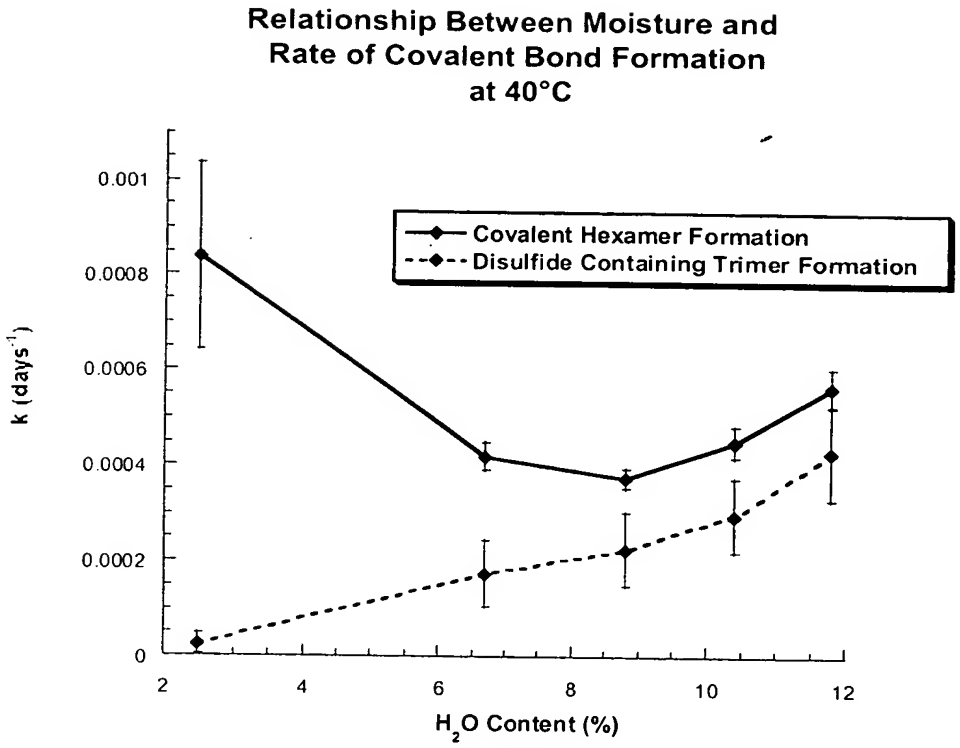

Relationship Between Moisture and Rate of Covalent Bond Formation at 50°C
Covalent Hexamer Formation
Disulfide Containing Trimer Formation
k (days-1)
0.0035
0.003
0.0025
0.002
0.0015
0.001
0.0005
0
2
4
6
8
10
12
H2O content (%)

Figure 19B


Relationship Between Moisture and
Rate of Covalent Bond Formation
at 40°C
Covalent Hexamer Formation
Disulfide Containing Trimer Formation
k (days-1)
0.001
0.0008
0.0006
0.0004
0.0002
0
2
4
6
8
10
12
H2O Content (%)